Comparison of CD271 (Adapalene) and All-*Trans* Retinoic Acid in Human Skin: Dissociation of Epidermal Effects and CRABP-II mRNA Expression

Christopher E.M. Griffiths,* James T. Elder,* Bruno A. Bernard,† Patricia Rossio,† Matthew A. Cromie,* Lawrence J. Finkel,* Braham Shroot,† and John J. Voorhees*

*Department of Dermatology, University of Michigan Medical Center, Ann Arbor, Michigan, U.S.A.; and †Centre International de Recherches Dermatologiques Galderma (CIRD Galderma), Sophia Antipolis, Valbonne, France

A new synthetic retinoid analogue, adapalene (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid, CD271), which is relatively selective for retinoic acid receptor β, was noted to be an effective comedolytic agent in the rhino mouse model and to have clinical efficacy against acne. In pursuit of this observation, we studied the effects of CD271 on the development of erythema, spongiosis, and epidermal hyperplasia as well as other well-characterized markers of *in vivo* retinoid action after 4 d of occluded topical treatment. The objective of the study was to elucidate further those parameters associated with potential clinical efficacy. Twenty-five subjects were treated with 0.1% all-*trans* retinoic acid cream, all-*trans* retinoic acid vehicle, 0.1% CD271 gel, or CD271 vehicle under occlusion for 4 d.

Only all-*trans* retinoic acid induced erythema ($p < 0.01$ versus all other treatments). Similarly, histologic analysis revealed that epidermal hyperplasia and spongiosis were induced only by all-*trans* retinoic acid ($p < 0.01$ versus all other treatments). By immunohistochemical analysis: all-*trans* retinoic acid increased expression of epidermal transglutaminase, involucrin, and calgranulin ($p < 0.05$ versus all other treatments). In contrast to these data, both CD271 and all-*trans* retinoic acid caused marked and significant ($p < 0.05$) elevation of cellular retinoic acid–binding protein-II (CRABP-II) messenger ribonucleic acid steady-state levels as judged by quantitative RNA blot analysis. Although CD271 treatment did not lead to erythema or affect epidermal morphology, its ability to induce a marker of retinoid action (i.e., CRABP-II) was 70% the potency of all-*trans* retinoic acid.

This study suggests that CRABP-II gene expression may be a more sensitive indicator of retinoid biologic activity in skin than are erythema or changes in epidermal morphology and differentiation.

Key words: epidermal hyperplasia/spongiosis/transglutaminase/calgranulin. *J Invest Dermatol 101:325–328, 1993*

Topical all-*trans* retinoic acid (RA) and other retinoids elicit an increasingly well-characterized series of clinical, histologic, cellular, and molecular responses when administered systemically or applied topically to human skin [1–4]. A similar spectrum of activities is observed after RA treatment of skin-equivalent cultures [5], demonstrating that retinoids are capable of profound and direct modulation of the intrinsic program of epidermal differentiation. Adapalene (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid, CD271) displays potent retinoid activity in several *in vitro* and *in vivo* assays [6,7], and shows relative selectivity for retinoic acid receptor β (RAR-β) and to a lesser extent RAR-γ [8]. In recent clinical studies, CD271 0.1% gel was shown to be as effective as 0.025% RA gel in the treatment of comedonal acne [9].

Murine and *in vitro* models of retinoid activity can supply information that, at times, is paradoxical to observations made *in vivo* in humans [10]. As a result of this conflict we have developed an *in vivo* human assay for epidermal retinoid activity that entails 4-d occlusion of the retinoid under study on the buttock skin of normal volunteers [1]. Changes in epidermal morphology produced by RA (spongiosis, epidermal hyperplasia, and thickening of the granular layer) at 4 d in this assay are similar to observations made after long-term clinical treatment [3,4]. Markers of differentiation and proliferation, such as epidermal transglutaminase and involucrin, are also induced by RA in this assay, again correlating with observations made after long-term treatment [2,11]. Cellular retinoic acid–binding protein-II (CRABP-II) ribonucleic acid, messenger (mRNA) is constitutively expressed in human and murine skin [12–14] but its expression is significantly enhanced after topical RA treatment [5,12]. CRABP-II is an early, specific, highly sensitive marker of topical retinoid activity, not merely associated with a hyperproliferative state [15].

In this study we have used a 4-d bioassay to compare the effects of 0.1% CD271 gel and 0.1% RA cream on erythema, epidermal morphology, markers of differentiation and CRABP-II mRNA expression.

Manuscript received November 18, 1992; accepted for publication April 12, 1993.

Supported in part by the Babcock Dermatologic Endowment, Ann Arbor, Michigan.

Bruno A. Bernard's current address: L'Oreal, Centre de Recherche Charles Zviak, Clichy, France.

Reprint requests to: Dr. C.E.M. Griffiths, Department of Dermatology, University of Michigan Medical Center, 1910 Taubman Center, Ann Arbor, MI 48109–0314.

Abbreviations: CD271, adapalene (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid); CRABP, cellular retinoic acid–binding protein; RA, all-*trans* retinoic acid; RAR, retinoic acid receptor.

0022-202X/93/$06.00

Table I. Comparison of Epidermal Histology Following 4-day Occlusive Treatment[a]

Histologic parameter[b]	RA vehicle (n = 9)	0.1% RA (n = 9)	CD271 vehicle (n = 9)	0.1% CD271 (n = 9)
Stratum corneum compaction	1.7 ± 0.4[c]	2.7 ± 0.4[e]	0.7 ± 0.3[d]	0.6 ± 0.2[d]
Granular layer thickness	0.7 ± 0.2[c]	2.0 ± 0.3[d]	0.2 ± 0.1[c]	0.6 ± 0.1[c]
Spongiosis	1.5 ± 0.3[c]	2.5 ± 0.2[d]	1.1 ± 0.3[c]	0.9 ± 0.3[c]
Mitoses/5 HPF	0.2 ± 0.2[c]	2.2 ± 0.7[d]	0.3 ± 0.2[c]	0.2 ± 0.2[c]
Epidermal thickness (μm)	61 ± 6[c]	100 ± 7[d]	53 ± 7[c]	51 ± 6[c]

[a] All values are mean ± SEM.
[b] Except for epidermal thickness and mitotic figures, all parameters are scored on a 0 to 4 scale.
[c,d,e] For each parameter, pairwise comparison of means with at least one superscript in common denotes non-significant differences at the 0.05 level.

MATERIALS AND METHODS

Normal volunteers (all of whom had given written consent in a protocol approved by the University of Michigan Medical Center Institutional Review Board) had the four test agents applied to buttock skin (150 mgs/9 cm^2) according to a computer-generated randomization code, and the sites occluded under Saran Wrap for 4 d. The test agents applied were 0.1% RA cream, RA vehicle cream (Ortho Pharmaceutical Corporation, Raritan, NJ), 0.1% CD271 aqueous gel suspension, and CD271 gel vehicle (Owen Galderma, Fort Worth, TX).

After 4 d, the occlusive patch was removed and the degree of erythema present at each test site scored according to a 10-point scale where 0 is none; 1–3, mild; 4–6, moderate, and 7–9, severe. Keratomes and 6-mm punch biopsies were performed under 1% plain lidocaine anesthesia.

In 10 subjects, two 6-mm punch biopsies were taken from each test site. One biopsy was placed in 10% neutral-buffered formalin and processed for hematoxylin and eosin histology and the other biopsy frozen in liquid nitrogen and stored at −70°C until used for immunohistochemical analysis. In eight subjects, a keratome biopsy (0.2 mm in depth) was taken from each treatment site, immediately snap-frozen in liquid nitrogen and stored at −70°C until used for RNA isolation.

Histology Biopsies processed to hematoxylin and eosin were read at the light microscope level by one investigator (LJF) who was blinded to which treatment group the specimen had come from. Stratum corneum compaction, spongiosis, and granular layer thickness were assessed using a 0 to 4 ordinal scale where 0 = none and 4 = maximum. Epidermal thickness measured in micrometers from the base of the stratum corneum to the basement membrane was assessed in five high power fields and the mean thickness used. Mitotic figures were counted per five high-power fields.

Immunohistochemistry Indirect immunofluorescence on 5-μm frozen sections was performed as previously described [16] using the following antibodies: F12 monoclonal antibody to calgranulin [17,18] and B.C1 monoclonal antibody to human transglutaminase [19] (generous gifts of Professor L. Juhlin, Uppsala, Sweden and Dr. S. Thacher, Texas A & M University, College Station, TX, respectively); mouse monoclonal antibody to human keratin 10 (RKSE60, Sanbio Laboratories, Netherlands) and rabbit polyclonal antibody to human involucrin (Biomedical Technologies, Inc., Stoughton, MA). A polyclonal antiserum to loricrin was obtained by immunizing a rabbit with a loricrin carboxyterminal peptide [20]. Fluorescein and rhodamine conjugates were purchased from Nordic, Netherlands.

Quantification of immunohistochemical staining reactions was as follows. For epidermal transglutaminase, involucrin, calgranulin, and loricrin, the number of epidermal cell layers staining for each marker was counted. In the case of keratin 10, the staining pattern was assessed as 0, strictly suprabasal; 1, minimum focal reduction; and 2, maximum focal reduction.

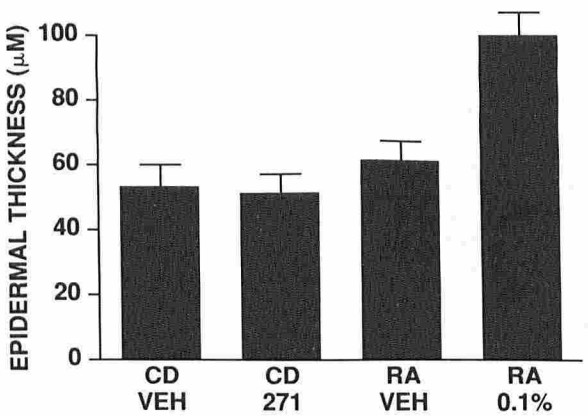

Figure 1. Epidermal thickness of 4-d occlusive treatment of normal skin with 0.1% RA cream, RA vehicle, 0.1% CD271 gel, or CD271 gel vehicle. RA produced significant ($p < 0.01$) epidermal hyperplasia as compared with the other three treatment, which were not significantly different from one another. All values mean ± SEM; n = 9.

RNA Isolation and Northern Blot Analysis Total RNA was isolated from snap-frozen keratome biopsies using the guanidinium-cesium chloride procedure as previously described [21], except that cesium trifluoroacetate (Pharmacia, Piscataway, NJ) was substituted for cesium chloride according to the manufacturer's instructions. Forty micrograms total RNA (determined by absorbance at 260 nm) was fractionated by electrophoresis over 1% formaldehyde-agarose gels and transferred to derivatized nylon membrane (Zeta-Probe, Bio-Rad, Richmond, CA). Blots were sequentially hybridized against ^{32}P-labeled CRABP-II and cyclophilin probes prepared by random priming and quantitated using a phosphorimager (Molecular Dynamics) as previously described [12]. Hybridization to the CRABP-II probe was normalized to cyclophilin and results for each patient expressed as number of times change relative to CD271 vehicle.

Statistical Methods Treatment group comparisons were assessed with a repeated measures analysis of variance and the Tukey multiple comparison test. Two-sided significance was determined at the 0.05 and 0.01 type I error rates. Summary data are represented as means ± SEM. The analyses were performed by BMDP statistical software.

RESULTS

Clinical Only RA induced erythema (score of 4.8 ± 0.3) and this was significantly more than CD271 (score of 0.3 ± 1) and all other treatments ($p < 0.01$; n = 25).

Histology RA increased epidermal hyperplasia (100 μm ± 7) as compared with RA vehicle (61 μm ± 6) and CD271 vehicle (53 μm ± 7) (see Table 1; n = 9 for all parameters). No epidermal hyperplasia was induced by CD271 (51 μm ± 6), and this was significantly less than RA ($p < 0.01$, Fig 1). Spongiosis was also significantly increased by RA (2.5 ± 0.2) as compared with CD271 (0.9 ± 0.3, $p < 0.01$).

Immunohistochemistry As previously observed in this bioassay system, the number of epidermal cell layers expressing epidermal transglutaminase was considerably increased following RA treatment (9.9 ± 1.0) as compared with vehicle (2.2 ± 0.2, $p < 0.01$) (see Table II; n = 10 for all parameters). Treatment with CD271 produced no expansion of epidermal transglutaminase staining and was not significantly different from vehicle. Both calgranulin and involucrin expressions were also enhanced by RA and these were significantly greater than CD271 ($p < 0.01$). Keratin 10 was focally reduced by RA as compared with a strictly suprabasal expression in vehicle-treated epidermis ($p < 0.05$), CD271 produced some reduction in keratin 10 expression but this was not significantly different from either vehicle. Loricrin expression was not significantly altered by any of the treatments.

CRABP-II mRNA The results of Northern blot analysis of tissue from two representative volunteers are shown in Fig 2 and data for the eight subjects studied are summarized in Fig 3. Although some variability in response to CD271 and RA vehicles was noted (compare *left* and *right panels,* Fig 2), a consistent, marked and signif-

Table II. Effects of 4-Day Occlusive Treatment on Markers of Differentiation[a]

Marker[b]	RA vehicle (n = 10)	0.1% RA (n = 10)	CD271 vehicle (n = 10)	0.1% CD271 (n = 10)
Keratin 10	0.3 ± 0.1[c]	1.0 ± 0.2[d]	0.1 ± 0.1[c]	0.4 ± 0.2[c,d]
Transglutaminase	2.2 ± 0.2[c]	9.9 ± 1.0[d]	1.9 ± 0.1[c]	2.1 ± 0.2[c]
Involucrin	4.2 ± 0.4[c]	13.0 ± 0.6[d]	3.0 ± 0.2[c]	3.4 ± 0.2[c]
Loricrin	2.4 ± 0.5[c]	2.5 ± 0.6[c]	3.1 ± 0.4[c]	2.9 ± 0.3[c]
Calgranulin	0.3 ± 0.2[c]	6.1 ± 1.1[d]	0.0 ± 0.0[c]	0.2 ± 0.1[c]

[a] All values are mean ± SEM.
[b] Keratin 10 is scored on a 0 to 2 scale (see *Materials and Methods*); all other values represent number of epidermal cell layers expressing the marker in question.
[c,d,e] For each marker, pairwise comparison of means with at least one superscript in common denotes non-significant differences at the 0.05 level.

icant induction of CRABP-II mRNA was noted in response to RA and CD271 relative to either vehicle ($p < 0.05$, Fig 3). CD271 increased CRABP-II mRNA to 70% of the levels observed with RA. By contrast, erythema and histologic effects were seen only with RA, CD271 and both vehicles having no significant effect.

DISCUSSION

This study demonstrates that CD271 is capable of inducing CRABP-II mRNA in the absence of changes in epidermal morphology. These findings contrast with the concordance observed between epidermal changes and CRABP-II expression following the administration of 0.1% RA cream.

The induction of epidermal hyperplasia and spongiosis, coupled with enhanced expression of epidermal transglutaminase and involucrin are in agreement with previous data using 0.1% RA cream in the 4-d assay [1,10,11]. However, it is unknown why RA focally inhibited keratin 10 and had no effect on loricrin expression in this study, whereas keratin 10 was unaffected and loricrin inhibited in previous work [11]. Calgranulin is an intracellular, calcium-binding protein absent from normal epidermis but present in the epidermis of inflammatory dermatoses [22]; the presence of calgranulin may be independent of keratinocyte proliferation [23]. In this study, calgranulin expression was significantly enhanced in RA-treated epidermis; the exact nature of this enhancement is unknown but probably relates to keratinocyte hyperproliferation in this case [17].

Overall, these short-term events under occlusion are predictive of epidermal changes in long-term, unoccluded, RA-treated skin [11]. Although CD271 0.1% gel possesses retinoid activity in the rhino mouse model, as evidenced by epidermal hyperplasia and comedolysis [6,7], this does not appear to be the case using the 4-d human assay. It is possible that the discrepancy between assay systems may simply be one of greater penetration of CD271 through relatively thin murine epidermis, thereby resulting in higher epidermal concentrations of the drug than could be obtained in human skin. However, the observed induction of CRABP-II gene expression by CD271, up to 70% of that seen with 0.1% RA cream, argues for penetrance of small amounts of CD271 under these conditions. We have previously observed that elevated CRABP-II levels are a highly sensitive marker of retinoid activity [15]. Indeed, RA at a concentration of 0.001% in ethanol/propylene glycol vehicle will induce CRABP-II expression in the absence of significant changes in epidermal histology (Griffiths CEM, Elder JT, unpublished observations).

CD271 belongs to a new class of synthetic retinoic acid analogues that exhibit some selectivity for certain RARs. In the case of CD271, the relative selectivity is for RAR-β [8], a receptor that in the skin is predominantly expressed in dermis, and to a lesser extent RAR-γ [8], which is mainly expressed in epidermis [5]. Although we have previously discounted any direct role in CRABP-II in the biologic action of CD271 [24] it is of interest to note that the gene encoding CRABP-II contains a retinoic acid response element in the promoter region that is transactivated by both RAR-β and RAR-γ [25]. CRABP-II appears to regulate the bioavailability of RA by acting as both a trap for RA and a cofactor in its metabolic oxidation [26]. In view of their distinct structural differences, it is unlikely that CD271 would undergo the same catabolic oxidation as RA. However, we cannot rule out that CD271 could be metabolized in the viable epidermis (demethylation for example) and that CRABP-II plays an indirect role in that process.

Reports of the efficacy of 0.1% CD271 gel in the treatment of acne [9] indicate that the rhino mouse model of comedolysis can be a predictor of retinoid efficacy for acne therapy. Whether changes in

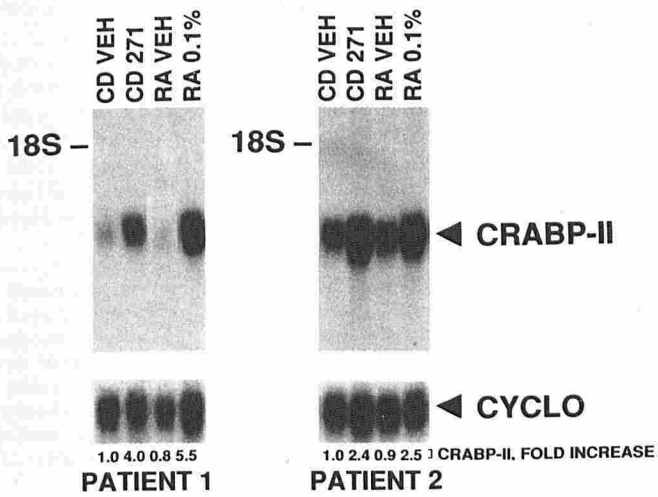

Figure 2. Northern blot of CRABP-II response to 4-d occlusive application of 0.1% RA cream versus 0.1% CD271 gel and respective vehicles. Results shown are from two representative patients in that they reflect the largest differences in signal observed over vehicle controls. Note that independent of the response to vehicle, CRABP-II is induced by both 0.1% RA and 0.1% CD271. Sizes of bands (in kb) are indicated to the left and probes used, CRABP-II and cyclophilin (cyclo), to the right.

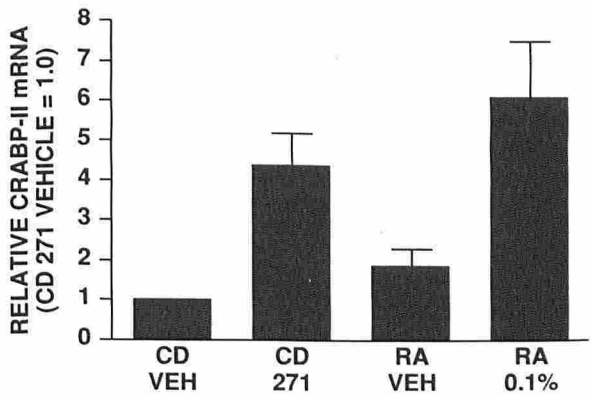

Figure 3. Quantitative analysis of Northern blots. Relative times increase in CRABP-II mRNA (relative to CD271 vehicle) following 4-d occlusive treatment of normal skin using 0.1% CD271 gel, 0.1% RA cream and RA vehicle. Both CD271 and RA produced a significant increase ($p < 0.05$) in CRABP-II mRNA levels relative to vehicle. All values are mean ± SEM; n = 8.

differentiation markers and epidermal hyperplasia in RA-treated skin are indicative of a therapeutic effect in acne and/or photodamage or are side effects is a point of considerable interest. Experiments performed in this assay with the irritant sodium lauryl sulfate indicate that, although an irritant will induce changes in epidermal morphology and markers of differentiation very similar to those produced by RA, the irritant does not elevate CRABP-II levels [15].

In conclusion, the 4-d human assay demonstrates that CD271, in a formulation known to be therapeutic in acne, does not induce the epidermal changes associated with retinoid activity but does elevate epidermal CRABP-II mRNA. The study underscores the sensitivity and specificity of CRABP-II elevation in response to topical retinoids whether it be a non-specific receptor agonist, such as RA, or a more selective agonist, as in the case of CD271. However, it still remains to be shown whether the ability to elevate CRABP-II mRNA can be a predictor of a retinoid's clinical efficacy.

We are grateful to Ted A. Hamilton, MS, for statistical analysis, Dale Yessian for secretarial assistance, and Robin Gardner for tissue procurement.

REFERENCES

1. Fisher GJ, Esmann J, Griffiths CEM, Talwar HS, Duell EA, Hammerberg C, Elder JT, Finkel LJ, Karabin GD, Nickoloff BJ, Cooper KD, Voorhees JJ: Cellular, immunological and biochemical characterization of topical retinoic acid-treated skin. *J Invest Dermatol* 96:699–707, 1991
2. Rosenthal DS, Roop DR, Huff CA, Weiss JS, Ellis CN, Hamilton TA, Voorhees JJ, Yuspa SH: Changes in photoaged human skin following topical application of all-*trans* retinoic acid. *J Invest Dermatol* 95:510–515, 1990
3. Weiss JS, Ellis CN, Headington JT, Tincoff T, Hamilton TA, Voorhees JJ: Topical tretinoin improves photoaged skin: a double-blind, vehicle-controlled study. *JAMA* 259:527–532, 1988
4. Bhawan J, Gonzalez-Serva A, Nehal K, Labadie R, Lufrano L, Thorne EG, Gilchrest BA: Effects of tretinoin on photodamaged skin: a histologic study. *Arch Dermatol* 127:666–672, 1991
5. Elder JT, Astrom A, Pettersson U, Tavakkol A, Griffiths CEM, Krust A, Kastner A, Chambon P, Voorhees JJ: Differential regulation of retinoic acid receptors and binding proteins in human skin. *J Invest Dermatol* 98:673–679, 1992
6. Bouclier M, Chatelus A, Ferracin J, Delain C, Shroot B, Hensby CN: Quantification of epidermal histological changes induced by topical retinoids and CD271 in the rhino mouse model using a standardized image analysis technique. *Skin Pharmacol* 4:65–73, 1991
7. Bernerd F, Ortonne J-P, Bouclier M, Chatelus A, Hensby C: The rhino mouse model: the effects of topically applied all-*trans* retinoic acid and CD271 on the fine structure of the epidermis and utricle wall of pseudocomedones. *Arch Dermatol Res* 283:100–107, 1991
8. Bernard BA, Bernardon JM, Delescluse C, Martin B, Charpentier B, Pilgrim WR, Reichert U, Shroot B: Identification of synthetic retinoids with selectivity for human nuclear synthetic retinoic acid receptor γ. *Biochem Biophys Res Commun* 186:977–983, 1992
9. Verschoore M, Langner A, Wolska H, Jablonska S, Czernielewski J, Schaefer H: Efficacy and safety of topical CD271 alcoholic gels. A new treatment candidate for acne vulgaris. *Br J Dermatol* 124:368–371, 1991
10. Griffiths CEM, Rosenthal DS, Reddy AP, Elder JT, Astrom A, Leach K, Wang TS, Finkel LJ, Yuspa SH, Voorhees JJ, Fisher GJ: Short-term retinoic acid treatment increases *in vivo*, but decreases *in vitro*, epidermal transglutaminase K enzyme activity and immunoreactivity. *J Invest Dermatol* 99:283–288, 1992
11. Rosenthal DS, Griffiths CEM, Yuspa SH, Roop DR, Voorhees JJ: Acute or chronic topical retinoic acid treatment of human skin *in vivo* alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10, and 14. *J Invest Dermatol* 98:343–350, 1992
12. Astrom A, Tavakkol A, Pettersson U, Cromie M, Elder JT, Voorhees JJ: Molecular cloning of two human cellular retinoic acid-binding proteins (CRABP). *J Biol Chem* 266:17662–17666, 1991
13. Eller MS, Oleksiak MF, McQuaid TJ, McAfee SG, Gilchrest BA: The molecular cloning and expression of two CRABP cDNAs from human skin. *Exp Cell Res* 199:328–336, 1992
14. Giguerre V, Lyn S, Yip P, Siu C-H, Amin S: Molecular cloning of cDNA encoding a second cellular retinoic acid-binding protein. *Proc Natl Acad Sci USA* 86:6233–6237, 1990
15. Elder JT, Cromie MA, Griffiths CEM, Chambon P, Voorhees JJ: Stimulus-selective induction of CRABP-II mRNA: a marker for retinoic acid action in human skin. *J Invest Dermatol* 100:356–359, 1993
16. Juhlin L, Juhlin C, Scheynius A, Klareskog L: Immunohistochemical reactivity of monoclonal antibodies generated after immunization of mice with cells from psoriatic lesions. *Acta Derm Venereol* 69:93–1001, 1989
17. Saintigny G, Schmidt R, Shroot B, Juhlin L, Reichert U, Michel S: Differential expression of calgranulin A and B in various cell lines and reconstructed epidermis. *J Invest Dermatol* 99:639–644, 1992
18. Thacher S, Rice RH: Keratinocyte-specific transglutaminase of cultured human epidermal cells: relation to cross-linked envelope formation and terminal differentiation. *Cell* 40:685–695, 1985
19. Magnaldo T, Bernerd F, Asselineau D, Darmon M: Expression of loricrin is negatively controlled by retinoic acid in human epidermis reconstructed in vitro. *Differentiation* 49:39–46, 1992
20. Bernard BA, Asselineau D, Schaffar-Deshayes L, Darmon M: Abnormal sequence of expression of differentiation markers in psoriatic epidermis: inversion of two steps in the differentiation program? *J Invest Dermatol* 90:801–805, 1988
21. Elder JT, Tavakkol A, Klein SB, Zeigler ME, Wicha M, Voorhees JJ: Protooncogene expression in normal and psoriatic skin. *J Invest Dermatol* 94:19–25, 1990
22. Kelly SE, Jones DB, Fleming S: Calgranulin expression in inflammatory dermatoses. *J Pathol* 159:17–21, 1989
23. Clark BR, Kelly SE, Fleming S: Calgranulin expression and association with the keratinocyte cytoskeleton. *J Pathol* 160:25:25–30, 1990
24. Asselineau D, Cavey M-T, Shroot B, Darmon M: Control of epidermal differentiation by a retinoid analogue unable to bind to cytosolic retinoic acid binding proteins (CRABP). *J Invest Dermatol* 98:128–134, 1992
25. Durand B, Saunders M, Leroy P, Leid M, Chambon P: All-*trans* and 9-*cis* retinoic acid induction of CRABP II transcription is mediated by RAR–RXR heterodimers bound to DR1 and DR2 repeated motifs. *Cell* 71:73–85, 1992
26. Fiorella PD, Napoli JL: Expression of cellular retinoic acid binding protein in Escherichia coli: characterization and evidence that holo-CRABP is a substrate in retinoic acid metabolism. *J Biol Chem* 266:16572–16579, 1991